Interstitial pH in human skeletal muscle during and after dynamic graded exercise

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1. In this study a new method has been used to measure interstitial pH continuously in human muscle during graded exercise. Human subjects performed 5 min of one-legged knee-extensor exercise at power outputs of 30, 50 and 70 W. Muscle interstitial pH was measured continuously in microdialysis dialysate using the pH-sensitive fluorescent dye 2′,7′-bis-(2-carboxyethyl)-5-(and -6)-carboxyfluorescein (BCECF).

2. The mean interstitial pH at rest was 7.38 ± 0.02. Interstitial pH gradually reduced during exercise in a nearly linear manner. The mean value (range) of the lowest interstitial pH at 30, 50 and 70 W exercise was 7.27 (7.18–7.34), 7.16 (7.05–7.24) and 7.04 (6.93–7.12), respectively.

3. The lowest pH was obtained 1 min after exercise, irrespectively of the workload, after which interstitial pH recovered in a nearly exponential manner. The mean half-time for recovery was 5.2 min (range 4.1–6.1 min). The changes in interstitial pH exceeded the changes in venous blood pH.

4. The present study showed that interstitial pH decreased during exercise in relation to intensity. These pH changes could have implications for blood flow regulation as well as for modulations of membrane transport systems.

During muscle activity, accumulation of lactic acid and CO_2 will reduce cellular pH and subsequently interstitial pH due to acid efflux from the muscle cells. It has been proposed that the changes in interstitial pH during muscle activity may be an important signal in the regulation of blood flow. In accordance with this idea, acidosis has been demonstrated to have a direct vasodilatory effect on vascular smooth muscle cells. This effect of acidosis is probably mediated by reduced extracellular pH and a subsequent lowering of the intracellular calcium concentration (Aalkjær & Peng, 1997). Furthermore, it has been suggested that changes in pH may modulate vascular K^+ channels (Davies, 1990) and thereby influence blood flow (Quayle *et al.* 1997), and that acidosis may activate sensory nerve endings located in the muscle interstitium (Victor *et al.* 1988). In order to evaluate such modulatory effects, it is important to describe and quantify the exercise-induced interstitial pH changes in human muscle.

The exercise-induced changes in muscle pH have, so far, mainly been described from changes in venous blood pH (Sjøgaard *et al.* 1985; Juel *et al.* 1990; Bangsbo *et al.* 1993*a*), but the relationship between interstitial pH and blood pH cannot be easily predicted because venous blood is mixed with blood draining inactive tissue (Rådegran & Saltin, 1998). Furthermore, studies using microdialysis in active muscle have demonstrated that during muscle activity interstitial concentrations of lactate were higher than venous lactate concentrations (MacLean *et al.* 1999), suggesting that the equilibration across the capillary wall is restricted. It may, therefore, also be expected that interstitial to venous pH gradients exist during exercise. In addition, the buffer capacity of interstitium and blood is different (Aukland & Reed, 1993). For these reasons, it can be hypothesised that the exercise-induced changes in interstitial and venous blood pH are different and that the changes in venous blood pH underestimate the local interstitial pH changes. Although electrodes have previously been used in an attempt to measure pH directly (Allsop *et al.* 1990), this was only performed at rest after exercise and showed an artificially slow recovery of muscle pH after muscle activity. Thus, it has not previously been possible to record interstitial pH during exercise in humans.

Therefore, the aim of the present study was to determine the changes in interstitial pH in human skeletal muscle during and after exercise at different intensities. For that purpose, a microdialysis technique was combined with the use of the pH-sensitive fluorescent dye BCECF, making it possible to perform continuous measurements of interstitial pH both during and after muscle activity.

METHODS

Subjects

Six male subjects with a mean age of 31 years (range 24–52 years) participated in the study. Mean height and weight were 181 cm (175–190 cm) and 82 kg (68–97 kg), respectively. All subjects were active individuals with no health-related problems. Prior to the start of the experiment, each subject was informed of any risks and discomforts of the experiment. All subjects signed a written consent form prior to experiments. The study was approved by the local ethics committee and conformed to the Declaration of Helsinki.

Exercise protocol

Subjects performed one-legged knee-extensor exercise in a supine position and were secured via a series of two shoulder, one waist and one thigh strap, so exercise was restricted to the quadriceps muscle (Bangsbo *et al.* 1990). During exercise the subjects had a visual feedback in the form of a digital display showing the cadence and power output. Subjects were required to maintain a cadence of 60 r.p.m. for 5 min duration at each work load. The subjects exercised at power outputs of 30, 50 and 70 W in random order and separated by 20 min of rest. Verbal encouragement was given to the subject at the higher intensities.

Probe insertion and perfusate

Before the experiment the subjects rested in a supine position with the legs well supported. For each microdialysis probe to be inserted, the subject was given approximately 1 ml of 20 g l^{-1} xylocaine via a 25-gauge needle at the insertion site. An 18-gauge cannula was first passed through the skin and fascia to make way for the probe. A second cannula containing the microdialysis probe (CMA-60, CMA Microdialysis AB, Sweden) was then pushed through the skin and fascia and orientated along the length of the fibres of the vastus lateralis. The cannula was removed leaving the microdialysis probe within the muscle. After insertion of the probe it was secured with tape and the outlet cut at a maximal length of 10 mm from the skin. The subjects recovered for 1.5 h after probe insertion before any measurements were performed.

The pH-sensitive fluorescent dye 2′,7′-bis-(2-carboxyethyl)-5-(and -6)-carboxyfluorescein (BCECF; no. D1880, Molecular Probes Inc.) was coupled to dextran (molecular mass 70 000 Da), which prevented any diffusion of dye across the probe membrane (cut off at a molecular mass of 20 000 Da). The dye (0.1 mg ml^{-1}) was dissolved in sterile saline solution (154 mM Na^+) to which 25 mM HCO_3^- was added without pH adjustment. The perfusate was placed into a sterile 1 ml syringe equipped with a filter, mounted in a microdialysis pump and connected to the inlet of the microdialysis probe. The outlet from the probe was removed, replaced with a steel tube (in order to avoid CO_2 diffusion) and connected to either a NanoFlo microfluorometer (World Precision Instruments) or a micro flow-through cuvette (total volume 8 μl) in a Hitachi F-2000 fluorescence spectrophotometer. The pump rate was 5 μl min^{-1} in all experiments. The time scales on the figures are corrected for the delay due to the volume of tubing and cuvette.

Fluorometric measurements and determination of pH

With the emission wavelength constant at 530 nm, the fluorescence spectrophotometer continuously switched between the excitation wavelengths of 440 and 500 nm with a bandpass of 10 nm. The excitation intensity at 440 nm was insensitive to pH, but dependent on the amount of dye, whereas the excitation intensity at 500 nm was also sensitive to pH. Thus, the excitation intensity ratio 500 nm/440 nm was proportional to pH and independent of changes in dye concentration and therefore was insensitive to any water movements from or into the probe. The temperature in the fluorescence spectrophotometer was kept constant by circulating thermostatically controlled water.

For calibration, a microdialysis probe was placed in a beaker with magnetic stirring and connected to a pump and the fluorometer. The beaker contained saline and bicarbonate, and the pH was monitored with a laboratory pH meter. The pH in the beaker was changed in a stepwise manner by adding HCl/NaOH and the excitation ratio was recorded. A calibration curve was obtained by plotting the excitation intensity ratio *versus* external pH. The constants obtained from a linear regression to the calibration curve were used to convert fluorescent signals obtained in human experiments to interstitial pH.

RESULTS

Interstitial pH at rest and during muscle activity

The mean interstitial pH at rest was 7.38 ± 0.02. Exercise induced a reduction in muscle interstitial pH in all six subjects and at all intensities (Fig. 1). Interstitial pH was gradually reduced during the 5 min of exercise. The decrease in interstitial pH during exercise was nearly linearly related to the power output. The mean value of the lowest interstitial pH at 30, 50 and 70 W exercise was 7.27 (range 7.18–7.34), 7.16 (7.05–7.24) and 7.04 (7.12–6.93), respectively. The mean peak acidification during exercise at a power output of 30, 50 and 70 W was 0.11 (0.06–0.20), 0.22 (0.13–0.34) and 0.34 (0.22–0.41) pH units, respectively. For each subject there was a correlation between power output and peak acidification (Fig. 2). The large inter-individual variation in peak acidification was probably due to the large variation in relative work load.

Recovery from exercise

The peak acidification was obtained 1.0 min (0.5–2.0 min) after the cessation of exercise. Recovery from peak acidification proceeded in an exponential fashion (Fig. 1). The mean half-time for recovery of interstitial pH after 70 W exercise was 5.2 (4.1–6.1) min. For most subjects the pH curve in recovery after 70 W intersected the curve obtained after 50 W, indicating that the rate of pH recovery was higher after 70 W than after 50 W exercise.

DISCUSSION

This is the first study to continuously measure interstitial pH during and after muscle activity in humans. At each intensity, interstitial pH gradually reduced during the entire exercise period. Peak acidification was obtained approximately 1 min after cessation of exercise, after which interstitial pH recovered in an exponential manner. It was also demonstrated that interstitial pH is reduced proportional to power output during muscle exercise.

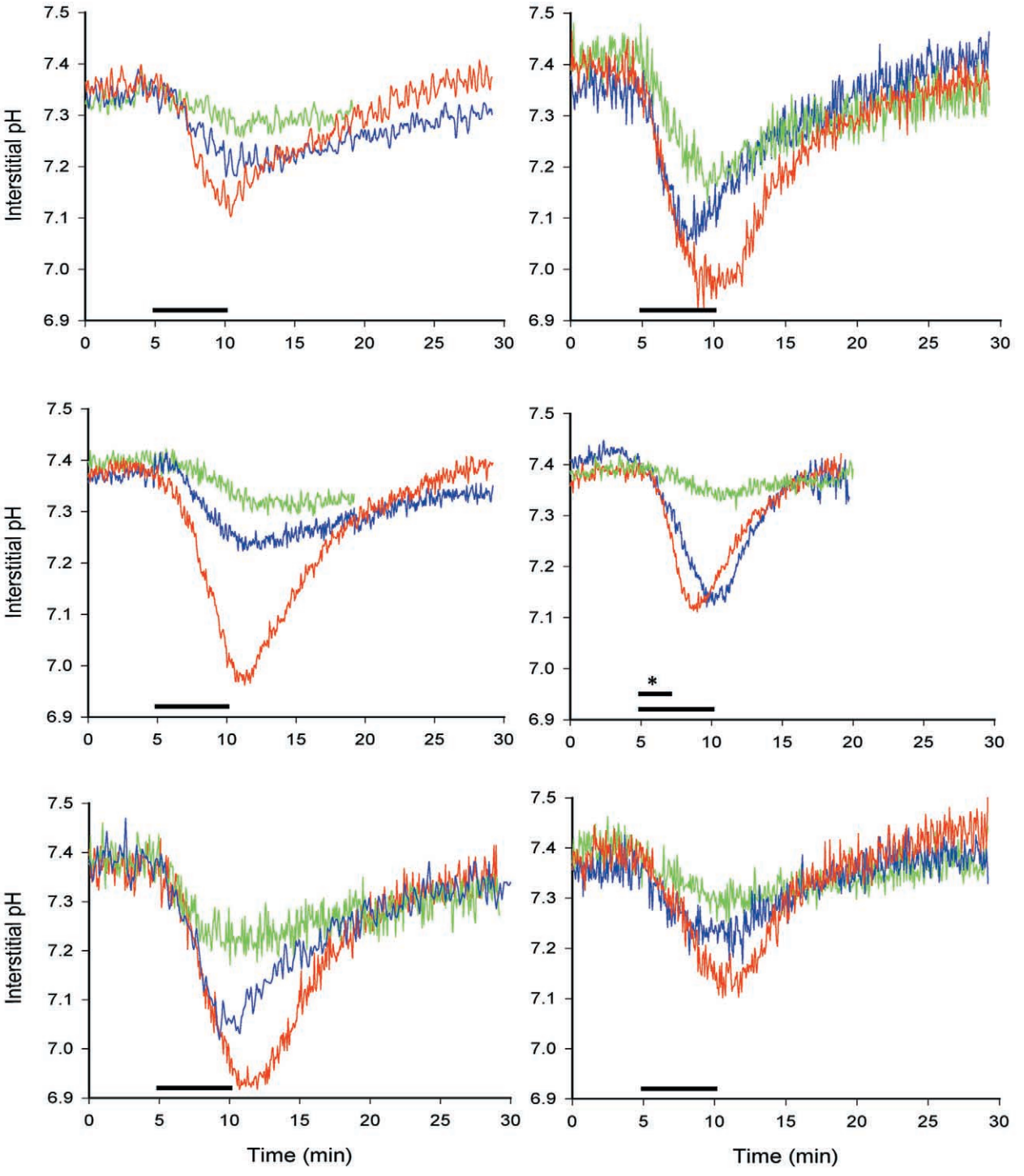

Figure 1. Interstitial acidification during exercise

Individual recordings of interstitial pH during 5 min of knee-extensor exercise are shown. The six panels refer to the six different subjects. The power output was 30 W (green), 50 W (blue) and 70 W (red). Exercise was started 5 min after onset of the recording (marked with horizontal bar). One subject, marked ∗, became exhausted after 2 min of 70 W exercise.

Changes in muscle interstitial pH during and after exercise

The rate of interstitial acidification at the onset of exercise can be evaluated from the slope of the curves in Fig. 1. In most subjects, the slope of acidification was greater the higher the power output. This finding probably reflects that the rate of acid accumulation in the muscle cells is related to the power output and that the acid transport across the sarcolemma membrane is dependent on the concentration gradient from muscle to interstitium.

The present study showed that muscle activity induced an interstitial acidification. In contrast, two studies also using the microdialysis technique but with the probe attached to a flow-through pH microelectrode have reported an alkalisation during muscle activity and a fast (1 min) recovery (MacLean *et al.* 1998, 2000). In these studies, no bicarbonate was added to the perfusate. When we did not add bicarbonate to the perfusate, we observed, as in previous studies (MacLean *et al.* 1998, 2000), that exercise induced an apparent fast alkalisation, succeeded by a plateau and a fast recovery (data not shown). It is likely that the large gradient of bicarbonate between interstitium and the probe had given rise to bicarbonate diffusing across the probe membrane. For most compounds the equilibration across the probe membrane is only partial (fractional uptake) and the rate of equilibration is increased by movement (MacLean *et al.* 1999; Juel *et al.* 2000). Therefore, the apparent alkalisation during exercise in their experiments could be due to an increased fractional uptake of bicarbonate induced by movement. Approximately 25 mM bicarbonate is present in blood (Bangsbo *et al.* 1997) and presumably also in the interstitium (Geers & Gros, 2000). Therefore, in the present study 25 mM bicarbonate was added to the perfusate, which resulted in a low bicarbonate concentration gradient between probe and interstitial space. With bicarbonate in the probe, exercise induced an interstitial acidification, succeeded by a recovery phase with a time course similar to recovery in the intracellular space and in blood (see below). It therefore appears that adding bicarbonate to the perfusate is crucial and that there is no evidence for an interstitial alkalisation during muscle activity.

Extracellular (interstitial) muscle pH has also been measured with a needle-protected glass pH electrode before and after exercise (Allsop *et al.* 1990). With this method pH after exercise was found to be 6.6. An apparently fast initial recovery after activity was succeeded by a slow, nearly linear recovery, which was only partial even after 30 min (Allsop *et al.* 1990). The low pH and the slow and only partial recovery could indicate that the measurements were influenced by fibre damage and/or the existence of a large artificial space in the muscle created by the needle. In contrast, the present study showed a complete recovery of interstitial pH 20 min after exercise.

Recovery of interstitial pH

The lowest interstitial pH was obtained approximately 1 min after exercise. A similar time course has been found for cellular pH determined by phosphorus magnetic

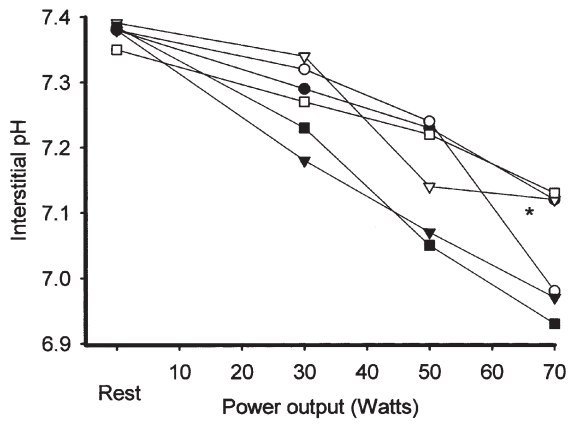

Figure 2. Peak interstitial pH during exercise at different power outputs

For each subject the line connects pH at rest and pH at peak interstitial acidification obtained at a power output of 30, 50 and 70 W. One subject, marked *, became exhausted after 2 min of 70 W exercise.

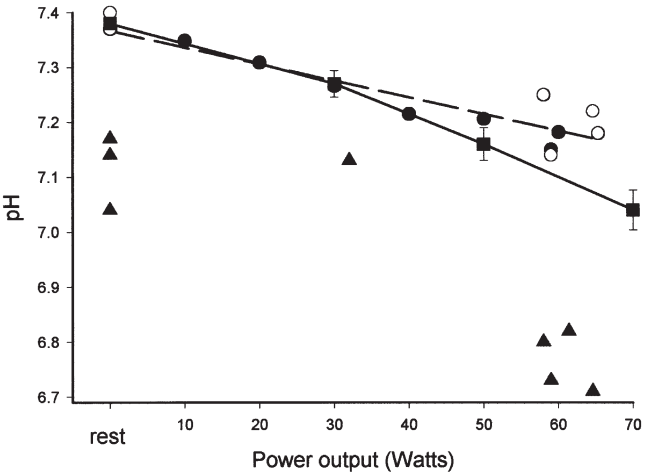

Figure 3. Intracellular, interstitial and venous pH during knee-extensor exercise

■, mean (± S.E.M.) peak interstitial pH obtained in the present study. Data connected by a straight line. ●, femoral venous pH during knee-extensor exercise (authors' unpublished data); each value represents mean from six subjects. ○, femoral venous pH during exhaustive knee-extensor exercise in three studies (Sjøgaard *et al.* 1985; Bangsbo *et al.* 1996, 2000). The dashed line represents a regression line for the venous data. ▲, intracellular pH obtained with the homogenisation technique (Sjøgaard *et al.* 1985; Juel *et al.* 1990; Bangsbo *et al.* 1993*a*, 1996). Data from the literature represent means of five to six subjects.

resonance spectroscopy (NMR) (Arnold *et al.* 1985; Bangsbo *et al.* 1993*b*). The reason for the further acidification after cessation of exercise is probably that a large fraction of phosphocreatine resynthesis, which releases H^+, occurs within the first minute after exercise (Arnold *et al.* 1985; Bangsbo *et al.* 1993*b*).

The recovery of interstitial pH took place with a mean half-time of 5.2 min calculated from the end of exercise (Fig. 1). This value is similar to the recovery of muscle pH (intracellular pH) measured in needle biopsies obtained at different time intervals after exercise (Juel *et al.* 1990; Juel, 1998). These studies have reported half-time values of 4.9 min for muscle pH, approximately 4 min for H^+, and 5 min for lactate (Juel *et al.* 1990; Juel, 1998). Thus, the time course of changes in interstitial pH after exercise is in good agreement with the changes in intracellular pH reported in the literature. In most of the subjects, the recovery of interstitial pH after intense exercise (70 W) was faster than after more moderate intensity exercise (50 W) (Fig. 1). In fact, some of the recovery curves for 70 W even crossed the curves for 50 W approximately 5 min after exercise. This observation could suggest that pH recovery is blood flow dependent, as blood flow after intense exercise is higher than after moderate intensity exercise (Rådegran & Saltin, 1998).

Comparison between pH changes in muscle interstitium and blood

The interstitial pH at rest (7.38 ± 0.02) fell within the range (7.37–7.43) of femoral venous pH values reported in the literature (Sjøgaard *et al.* 1985; Juel *et al.* 1990; Bangsbo *et al.* 1993*a*; Bangsbo *et al.* 2000). In Fig. 3, the exercise-induced interstitial pH values at peak acidification are compared to femoral venous pH values. The figure includes unpublished values from this laboratory as well as femoral venous blood pH values obtained in comparable knee-extensor exercise studies (Sjøgaard *et al.* 1985; Bangsbo *et al.* 1993*a*, 2000). It can be concluded from the figure that for 10–30 W intensity exercise the amplitude of the exercise-induced interstitial acidification is similar to the acidification of venous blood, whereas for intense exercise (> 50 W) the interstitial acidification is larger than the acidification of venous blood. There may be several reasons for this difference.

During one-legged knee-extensor exercise the femoral vein does not drain only the active part of the quadriceps muscle, since a fraction of venous blood comes from inactive tissues and inactive parts of the quadriceps muscle. At an exercise intensity of 50 W, blood flow may be $5.3\ l\ min^{-1}$ (Juel *et al.* 1990; Bangsbo *et al.* 1990) of which $1.2\ l\ min^{-1}$ may be perfusing inactive muscle (Rådegran & Saltin, 1998). This will have the effect that the decrease in femoral venous blood pH is less than the pH decrease in the interstitium of the active muscle. Another reason for the larger acidification of the interstitial space during muscle activity is probably its lower buffer capacity compared to blood. The protein concentration in the interstitial space is approximately half of the concentration in blood (Aukland & Reed, 1993), which makes the interstitium more exposed to pH fluctuations. A third possibility is that the reduced mean transit time for the blood passing the capillaries in the active muscle during exercise (Bangsbo *et al.* 1990; Juel *et al.* 1990; Rådegran & Saltin, 1998) may result in only partial equilibration of H^+ between interstitium and blood. The present study does not allow a discrimination between the possibility that a pH gradient exists across the capillary wall during exercise and that the pH difference between interstitium and femoral venous blood is exclusively due to mixing with blood from inactive tissues.

Comparison between cellular and interstitial pH changes

One method to access intracellular pH has been to measure pH in homogenised needle-biopsy material. This method is based on the fact that the intracellular space makes up the main fraction of the muscle and that the homogenate pH therefore mainly represents the intracellular pH (Sjøgaard *et al.* 1985). With the homogenisation method, intracellular pH values in the range 7.04–7.17 have been obtained for resting muscle (Sjøgaard *et al.* 1985; Juel *et al.* 1990; Bangsbo *et al.* 1993*a*; Bangsbo *et al.* 1996). Thus resting muscle pH is at least 0.2 pH units lower than interstitial pH (7.38).

In Fig. 3, the intracellular pH values during exercise reported in the literature are plotted together with the interstitial pH values obtained in the present study. It can be seen that cellular pH is 0.2–0.3 pH units lower than interstitial pH both at rest and immediately after intense exercise. However, if the data in the figure are converted to concentration of H^+ it is evident that the H^+ gradient across the muscle membrane is larger (approximately 100 nM) after intense exercise than the gradient at rest (30 nM). This is probably due to the large intracellular accumulation of lactic acid during muscle activity (Juel *et al.* 1990).

Physiological implications of changes in interstitial pH

The relation between exercise intensity and changes in interstitial pH showed similarities with exercise-related changes in blood flow (Juel *et al.* 1990; Bangsbo *et al.* 1990). It is therefore tempting to speculate that blood flow during muscle activity is regulated by changes in interstitial pH. In support of this hypothesis, interstitial pH has been reported to have a local modulatory effect on smooth muscle cells and cause vasodilatation, probably mediated by a reduction in intracellular calcium (Aalkjær & Peng, 1997) or changes in activity of potassium channels (Davies, 1990; Quayle *et al.* 1997).

On the other hand, blood flow during knee-extensor exercise increases rapidly and has reached a steady level after about 1.5 min (Rådegran & Saltin, 1998), whereas

interstitial pH progressively decreases during exercise. In addition, the time courses for recovery of blood flow and interstitial pH after exercise are different (Juel *et al.* 1990; Bangsbo *et al.* 1990). Therefore, other factors are also involved in blood flow regulation during and after exercise.

The decrease in interstitial pH in association with exercise may also have other effects. The lower interstitial pH could modulate the sensory response from the muscle, since pH in some studies has been shown to be coupled to sympathetic nerve discharge (Victor *et al.* 1988). Interstitial pH is probably also important in modulating transport systems and ion channels in muscle sarcolemma as well as in vascular cells.

Summary

The present study demonstrated that interstitial pH is continuously decreasing during muscle activity. The exercise-induced reduction in interstitial pH was correlated with power output and at high exercise intensities it was larger than the pH reduction of femoral venous blood.

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